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Short Communication

7-Substituted Pterins: Loading Experiments with 3'-Mono Deuterated Tetrahydro-L-Biopterin

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Introduction

Since 7-substituted pterins have been detected in the urine of patients with an atypical, transient form of hyperphenylalaninemia the origin of these metabolites has been unclear for a long time. The following pterins have been found so far: the 7-isomer of L-biopterin referred to as L-primapterin, the 7-isomer of D-neopterin referred to as D- or L-anapterin, and the 6-oxo-7-isomer of 7-oxo-L-biopterin referred to as 6-oxo-L- or D-primapterin (1).

Two findings make it plausible that these compounds derive from their 6-analogues: on the one hand, orally loading of a primapterinuric patient with L-tetrahydrobiopterin (L-BH₄) led to an increase of both L-biopterin and L-primapterin (2). And, *in vitro* incubation of L-tetrahydrobiopterin with pterin-4a-carbinolamine dehydratase-free phenylalanine hydroxylase results in the formation of L-primapterin (3, 4). Analogue *in vitro* incubation experiments with D-tetrahydroneopterin also led to the formation of the 7-substituted isomer.

For *in vivo* investigations of L-primapterin biosynthesis we loaded a primapterinuric patient with side-chain mono-deuterium labelled L-BH₄ ([3'-²H₁]-L-BH₄) (5). If L-primapterin originates from L-BH₄, deuterium labelling should also be present in L-primapterin isolated from the urine after loading, and it can also be determined whether the side-chain is from the same origin.

Material and Methods

Loading of a 5 year old male primapterinuric patient T. S. was performed with a dose of 10 mg deuterated L-BH₄ per kilogram body weight. His urine was collected up to 12 hours after loading.

The purification and isolation of urinary L-biopterin and L-primapterin after loading was performed using several different chromatographic and filtration steps: After acidic oxidation of the urine with iodine the acidic solution (0.1 N HCl) was applied to a Dowex 1 × 4 column. The column was washed with 0.1 N HCl and fractions fluorescing blue were collected. Purines were separated from pterins by precipitation in aqueous ammonia (pH 8–9). The alkaline solution was applied to a Dowex 50 × 8 column where pterins and salts passed through. The collected blue fluorescent fractions were made alkaline (pH 8–9) and then applied on a Dowex 1 × 4 column. Pterins were eluted with 0.1 N HCOOH. The eluate was made alkaline with NH₄OH and applied to a Dowex 1 × 4 column and the pterins were fractionated with a 0.1 N NH₄⁺COO⁻ buffer, pH 7.8. The pterin solution was made alkaline with NH₄OH (pH 8–9) and applied to a second Dowex 1 × 4 column. The column was washed with water, and the pterins were eluted with 0.1 N HCOOH. Excess acid was removed by evaporation, and the residue was diluted with water. The separation of the pterins was performed using a preparative reverse-phase HPLC column (6). After the first preparative HPLC-separation L-primapterin fractions still contained some L-biopterin. Therefore a second preparative HPLC was necessary.

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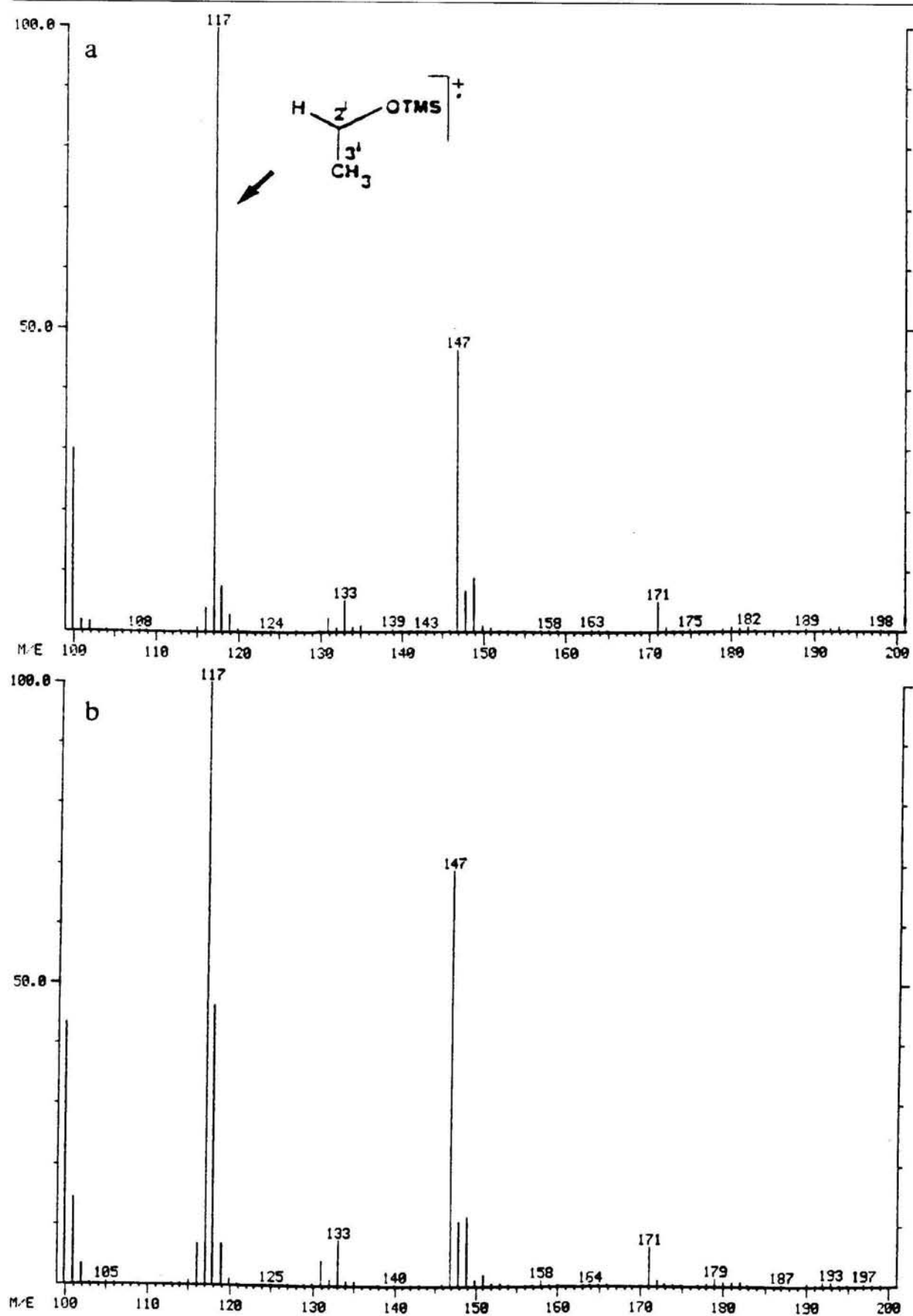


Figure 1. Low molecular fragments of L-primapterin. a) reference compound; b) isolated from urine after L-[3'- 2H] BH_4 -loading.

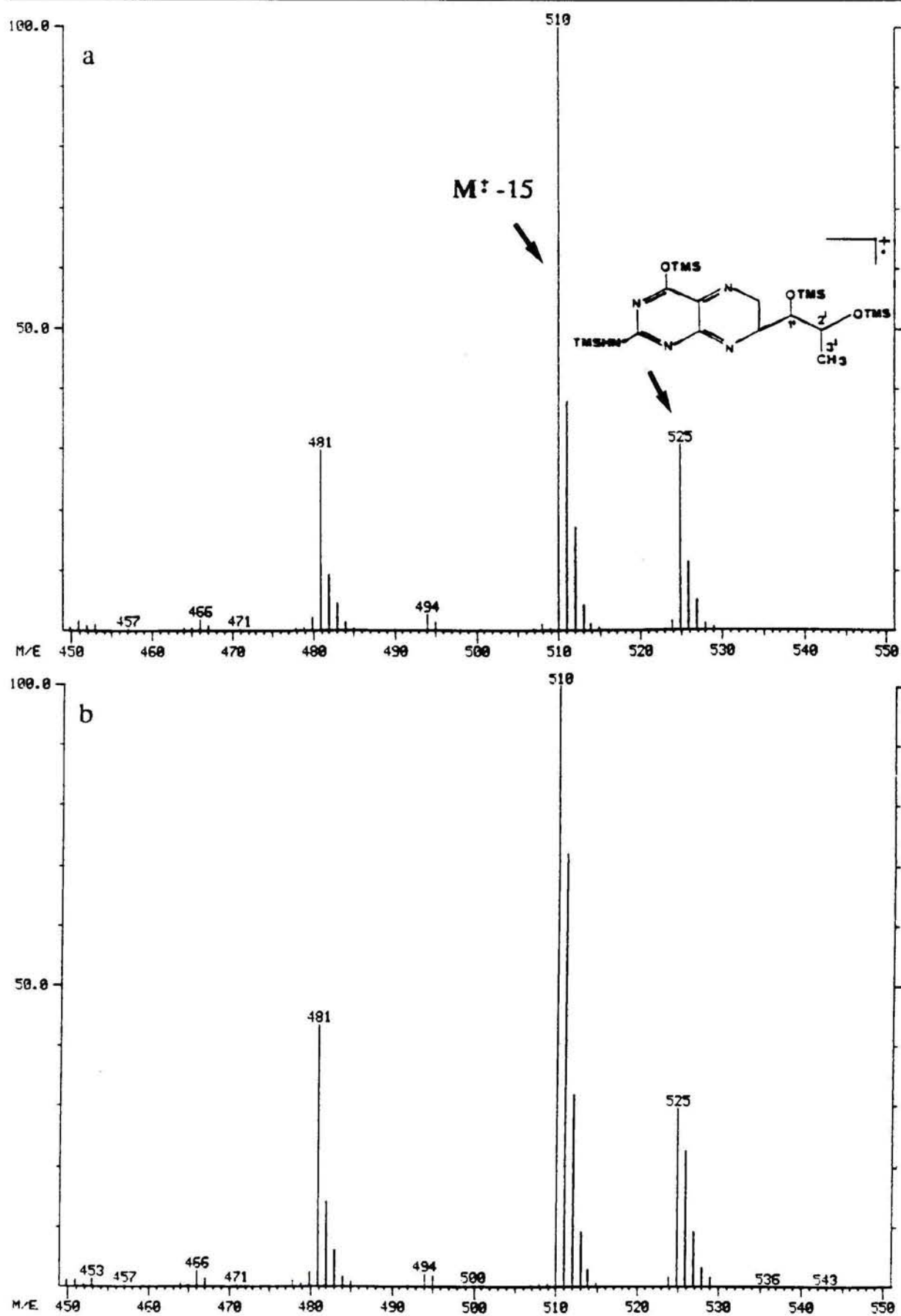


Figure 2. High molecular fragments of L-primapterin. a) reference compound; b) isolated from urine after L-[3'- 2 H₁]BH₄-loading.

Using the above described method of purification and isolation 300 µg of L-biopterin and 50 µg of L-primapterin were isolated from 200 ml urine.

Gas-chromatography/mass-spectrometry was performed using the TMS-derivatives of L-biopterin and L-primapterin (7).

Results and Discussion

We synthesized 3'-mono deuterated L-biopterin and analyzed it by GC/MS as a TMS-derivative. These data were compared with the MS data of unlabelled L-biopterin. Three ions were affected by a + 1 Da shift, i.e. m/z 117 containing the C-2',3'-end of the side-chain, m/z 510 originating from M^+-15 often observed for TMS-derivatives and the molecular ion at m/z 525.

L-biopterin as well as L-primapterin have the same electron impact-GC/MS fragmentation pattern, and differ only in their retention times on the GC column.

In the GC/MS spectrum of L-biopterin, isolated after the loading, a shift of + 1 Da was observed for the primary ions expected to exhibit deuterium incorporation, i.e. m/z 117, 510, and 525, were shifted to m/z 118, 511, and 526, respectively. Ion intensity values for m/z 118, 511, and 526 were found to have increased by 40% for this deuterium labelled sample relative to the standard (data not shown).

The same is true for isolated L-primapterin after loading with deuterated L-biopterin. Again, all three relevant ions (m/z 117, 510, and 525) are shifted

+ 1 Da (absolute intensity increase ~ 40%) indicative of deuterium incorporation (see Fig. 1 and 2).

This indicates clearly that the isolated L-primapterin originates from L-biopterin and, in addition, that the side-chain of L-primapterin derives from L-biopterin. With this experiment we can exclude an intermolecular reaction where the side-chain attaches to an already existing pterin ring nucleus.

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